

In the Specification:

Please replace the paragraph beginning on page 7, line 25 with the following new paragraph:

“Figure 1 shows the secretion of the C-HlyA polypeptide containing the ZIP domain. Figure 1A shows a schematic representation of the structure of the polypeptides EHlyA (SEQ ID NO: 1) and ZEHlyA (SEQ ID NO: 2) containing the 23 kDa ('hlyA) secretion signal of the *E. coli* Hly transporter tagged with the E epitope. The mass of said polypeptides (in kDa), deduced from its amino acid sequence, is shown to the right. The composition of the ZIP domain (Ig hinge, leucine zipper, 6xhis tag) is indicated. The amino acid sequence of the N-terminal region of both polypeptides EHlyA and ZEHlyA is also shown. Figure 1B shows a schematic representation of the polypeptide C-HlyA (monomeric) tagged with the E epitope and of the C-HlyA polypeptide (dimeric) tagged with the E epitope and containing the ZIP domain (Ig hinge, leucine zipper, 6xhis tag). Figure 1C shows the result of the immunoblotting carried out with a POD-labeled anti-E monoclonal antibody of the secreted (S) and cell (C) proteins produced after the 4 hour induction with 0.3 IPTG mM of *E. coli* HB2151 cell cultures, grown at 37°C, and one of the indicated plasmids, pEHlyA or pZEHlyA. The proteins loaded in each lane represent those found in about 5 µl of the supernatants (S) of the culture and those of the *E. coli* cells (C) present in about 100 µl of the same cultures (OD_{600nm} about 2).”

Please replace the paragraph beginning on page 10 , line 15 with the following new paragraph:

“Figure 6 shows the map of plasmid pZEHlyA. The sense (i.e. coding) strand is shown in SEQ ID NO: 3. The missense strand is shown in SEQ ID NO: 4. The protein sequence of the pZEHlyA plasmid is shown in SEQ ID NO: 5.”

Please replace the paragraph beginning on page 10, line 16 with the following new paragraph:

“Figure 7 shows the map of the plasmid pZEHlyA2-SD. The sense (i.e. coding) strand is shown in SEQ ID NO: 6. The missense strand is shown in SEQ ID NO: 7. The protein sequence of the pZEHlyA2-SD plasmid is shown in SEQ ID NO: 8.”

Please replace the paragraph beginning on page 10, line 17 with the following new paragraph:

“Figure 8 shows the map of the plasmid pV_{amy}HlyA. The sense (i.e. coding) strand is shown in SEQ ID NO: 9. The missense strand is shown in SEQ ID NO: 10. The protein sequence of the pV_{amy}HlyA plasmid is shown in SEQ ID NO: 11.”

Please replace the paragraph beginning on page 10, line 18 with the following new paragraph:

“Figure 9 shows the map of the plasmid pV_{amy}ZHlyA. The sense (i.e. coding) strand is shown in SEQ ID NO: 12. The missense strand is shown in SEQ ID NO: 13. The protein sequence of the pV_{amy}ZHlyA plasmid is shown in SEQ ID NO: 14.”

Please replace the paragraph beginning on page 12, line 30 with the following new paragraph:

“In a specific embodiment of the invention, said third nucleic acid sequence contains the nucleotide sequence identified as SEQ ID NO: 4 15 coding for a peptide of about 23 kDa of the carboxyl terminal end of *E. coli* HlyA, the amino acid sequence of which is shown in SEQ ID NO: 2 16.

Please replace the paragraph beginning on page 12, line 35 with the following new paragraph:

“Generally, the dimerization domain is not directly fused to the gene encoding the product of interest, but it is advantageous to introduce a spacer (flexible) peptide between the end of the gene coding for the product of interest and the beginning of the dimerization domain. Therefore, if so desired, the DNA construct of the invention can further contain a fourth nucleic acid sequence coding for a spacer peptide located between said first and second nucleic acid sequences, wherein the 5' end of said fourth nucleic acid sequence is bound to the 3' end of said first nucleic acid sequence, and the 5' end of said second nucleic acid sequence. In this manner the coding sequence of the product of interest is bound to the dimerization domain by means of a spacer peptide. Advantageously, said spacer peptide is a peptide with flexibility can be used. Virtually any peptide with structural flexibility can be used. As an example, said flexible peptide Gly-Gly-Gly-Ser, or any other suitable repetition of amino acid residues, or else the hinge region of an antibody. In a particular embodiment, said flexible spacer peptide comprises

the hinge region of antibody and the DNA construct of the invention contains the coding sequence for said flexible peptide. In a specific embodiment of the invention, said fourth nucleic acid sequence contains the nucleotide sequence identified as SEQ ID NO: 3 17 coding for a 10-amino acid peptide comprising the hinge region of an antibody the amino acid sequence of which is shown in SEQ ID NO: [[4]] 18 .”

Please replace the paragraph beginning on page 24, line 6 with the following new paragraph:

“Plasmids and oligonucleotides. Standard DNA handling and isolation methods, PCR amplification, and DNA sequencing were used [Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl 1994. Current Protocols in Molecular Biology. John Wiley & Sons, New York; Sambrook J., E. Fritsch, and T. Maniatis 1989. Molecular Cloning, a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York]. The oligonucleotides were obtained from Sigma Genosys (United Kingdom) or from Isogen Bioscience BV (Netherlands). The plasmids pEHlyA (Ap^r), pEHlyA2-SD (Ap^r) and pVDL9.3 (Cm^r) have already been described [Fernandez, L.A. and V. de Lorenzo. 2001. Formation of disulphide bonds during secretion of proteins through the periplasmic-independent type I pathway. Mol. Microbiol. 40:332-46; Fernandez, L.A., I. Sola, L. Enjuanes and V. de Lorenzo. 2000. Specific secretion of active single-chain Fv antibodies into the supernatants of *Escherechia coli* cultures by use of the hemolysin system. Appl. Environ. Microbiol. 66:5024-5029; Tzschaschel, B.D., C.A. Guzman, K.N. Timmis, and V. de Lorenzo 1996. An *Escherechia coli* hemolysin transport system-based vector for the export of polypeptides: Export of Shiga-like toxin IIeB subunit by *Salmonella typhimurium* aro4. Nature Biotechnology. 14:765-769]. The plasmid pZEHlyA (Ap^r) was obtained by inserting in the only pEHlyA *SalI* site a 170 bp DNA fragment coding for the ZIP domain amplified by PCR and digested with *SalI*. The map of the plasmid pZEHlyA is shown in Figure 6. PCR amplification of the ZIP domain was carried out with Vent DNA polymerase (New England Biolabs), using 1 ng of pCLZIP (Codon Genetic Systems, GmbH) as a template, and the oligonucleotides identified as SEQ ID NO: 5 19 and SEQ ID NO: 6 20, which incorporated two *SalI* sites flanking the amplified product, as primers. The plasmid pZEHlyA2-sd (Ap^r) was obtained by inserting in the only pEHlyA2-SD *SalI* site the 170 bp DNA fragment coding for ZIP obtained by digestion with pZEHlyA *SalI*. The map of plasmid pZEHlyA2-SD is shown in Figure 7. The orientation of the ZOP DNA fragment which produced an

internal insertion in the E-tagged C-HlyA domain of pZEHlyA and pZEHlyA2-SD after DNA sequencing was chosen. The DNA fragments of about 0.3 kb which encoded for the VHH, V_{amy} and V_{tx} domains were amplified by means of PCT with Vent DNA polymerase, using 1 ng of the A100R3A2 (anti- α -amylase) or R3E5 (anti-tetanus vaccine) phagemids, respectively, as a template and the oligonucleotides identified as SEQ ID NO: 7 21 and SEQ ID NO: 8 22 as primers.”